REMARKS

Claims 1-7 have been amended. Claims 8-61 have been withdrawn. Claims 62 and 63 have been added. Support for the amendment to claim 1 and for new claims 62 and 63 can be found, e.g., at page 9, lines 15-26, page 10, line 22, page 10, lines 24-25, and page 11, lines 19-21, of the specification

In paragraph 2 of the Office Action, the Examiner asked that Applicant identify all sequences with the appropriate SEQ ID NOs. The amendment to the specification inserts SEQ ID NOs after the sequences that lacked them in the application.

In paragraph 4 of the Office Action, the Examiner asked that a new title restricted to the claimed invention be provided. The title has been amended to "Hematopoietic Cell E-Selectin/L-Selectin Ligand Polypeptides," thereby addressing this objection. No new matter has been added.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 1-6 were rejected for lacking written description. In paragraph 7 of the Office Action, the Examiner stated

> Applicant relies upon identifying the KG1a CD44 glycoprotein by SEQ ID NO:1 and certain other features. However, there is insufficient written description of additional species of KG1a CD44 glycoproteins which are 95% similar to SEQ ID NO:1...In the absence of a nexus between structural and functional characteristics that are shared by members of the genus of KG1a CD44 glycoproteins, including those "95% similar to SEQ ID NO:1," one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus.

This rejection has been met, in part, and is traversed in part. The claims are directed to preparations of a substantially purified glycosylated CD44 polypeptide including an amino acid sequence at least 95% identical to SEQ ID NO: 1 or to a sequence of a CD44 isoform arising from alternative splicing, wherein the glycosylated polypeptide binds to an antibody having the binding specificity of monoclonal antibody HECA-452, and wherein the preparation comprises

less than 30% of a polypeptide other than the glycosylated CD44 polypeptide. As amended, the claims require 95% identity, rather than similarity, to SEQ ID NO:1, or to isoforms thereof arising from alternative splicing. Contrary to the Examiner's assertions, the disclosure provides multiple species and nexi between structural and functional characteristics shared by members of the genus.

The Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112 ¶ 1, "Written Description" Requirement, 66 Fed. Reg. 1099 (Jan. 5, 2001)("the Guidelines") state

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice...or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics. (Guidelines, page 1106, column 3, third paragraph).

Applicants have disclosed the relevant, identifying characteristics of the claimed genus. For example, one identifying characteristic of the claimed polypeptides is their ability to bind to an antibody having the binding specificity of monoclonal antibody HECA-452, which is for sialylated carbohydrate epitopes. Thus, one characteristic of the claimed polypeptides (binding to an antibody having a specificity of HECA-452) correlates directly with structural features of the genus; the members of the genus include sialylated carbohydrate and therefore include amino acid residues that can be glycosylated.

Moreover, Applicants direct the Examiner to Example 14 of the Revised Interim Written Description Guidelines Training Materials ("the Training Materials"). This Example describes a scenario in which a protein having at least 95% identity to a reference sequence, and a specific functional activity, is claimed. The Training Materials affirm the adequacy of the written description for this claim, even when only a single representative species is disclosed. The Training Materials state that the disclosure of a single protein species is sufficiently representative of the claimed genus because all members of the genus have high structural identity with the species, and because the Applicant provided an assay with which to identify variants having the recited high level of identity and the required functional property. The

present claims are supported by ample disclosed structural and functional information. The claims are directed to a genus having a very high degree of percent identity to a reference sequence and a required functional activity. As the claims provide at least as much written description support as provided in Example 14, the claims are adequately described.

Furthermore, a written description inquiry is made from the standpoint of one having skill and knowledge in the art. The support for sequences having 95% identity to SEQ ID NO:1 and isoforms thereof meets the standard for written description. As noted in the Guidelines, "in most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention" (Guidelines, page 1106, second column, third paragraph, citations omitted). At the time the present application was filed, the sequences of CD44 polypeptides having SEQ ID NO:1 and isoforms arising from alternative splicing were known. The degree of conservation in each region of the protein between isoforms and species had been well-characterized. For example, it was known that "the amino terminal ~180 aa are conserved among mammalian species (~85% homology). This region contains six conserved cysteines, and six conserved consensus sites for N glycosylation. Five conserved consensus sites for N-glycosylation are located in the amino terminal 120 aa of CD44" (specification, page 9, lines 28-31). Clearly, the knowledge of one of skill with regard to amino acid acid sequences of polypeptides of the claimed genus was considerable at the time of filing. Applicant asks that the rejection of claims 1-6 under 35 U.S.C. § 112, first paragraph, be withdrawn.

Enablement

Claims 1-6 were rejected as non-enabled. The Examiner stated

> Since the amino acid sequence of a polypeptide determines its structural and functional properties, predictability of which changes can be tolerated in a polypeptide's amino acid sequence and still retain a similar functionality (e.g., L-selectin ligand or E-selectin ligand) requires a knowledge of and guidance with regard to which amino acids, if any, are tolerant of modification and which are conserved...Because of lack of sufficient guidance and predictability in

> determining which modifications would lead to "95% sequence similar to SEQ ID NO:1"...was not well understood and was not predictable...it would require an undue amount of experimentation for one of skill in the art to arrive at enabling the genus of KG1a CD44 glycosylated polypeptides which are "95% similar to SEQ ID NO:1".

This rejection has been met, in part, and is traversed, in part. The claims have been amended to require that the glycosylated polypeptides comprise a sequence at least 95% identical to SEQ ID NO:1 or a sequence of a CD44 isoform arising from alternative splicing. This genus is fully enabled by the specification as filed. As discussed above, the level of knowledge required to enable the genus of polypeptides at least 95% identical to SEQ ID NO:1 and CD44 isoforms was high at the time of filing. For example, it was known which regions of the polypeptide were highly conserved (i.e., the amino terminal 180 amino acids, the six cysteines in and the six consensus sites for N-glycosylation within the amino terminal 180 amino acids) and which regions were non-conserved (amino acids 183-256). Thus, it does not require undue experimentation to arrive at polypeptides that fall within the claimed genus and that retain functionality (e.g., binding to an antibody having the specificity of HECA-452).

Ample guidance for features required for functionality such as L-selectin and E-selectin ligand activity is provided by the specification as filed. For example, Applicant has shown that both E-selectin and L-selectin ligand activities are sensitive to treatment with N-glycosidase F, and therefore are dependent on the presence of N-linked glycans (see, e.g., Example 3, page 42, lines 19-22 and Example 7, page 47, lines 21-23). It would not require undue experimentation to arrive at a polypeptide with 95% identity to a CD44 sequence that retained N-linked glycans, given the fact that consensus sites for N-linked glycosylation are known and are found in the claimed polypeptides. In view of the state of the art, the high degree of identity between the claimed sequences, the disclosure of multiple species that fall within the claimed genus, and the presence of working examples, Applicant submits that the claims are enabled.

Claims 1-7 were rejected as non-enabled on the grounds that the HECA-452 antibody is required to practice the claimed invention and thus, must be known and readily available. HECA-452 is a well known and publicly available antibody. As noted on page 10, line 13, of the

specification, the hybridoma that produces HECA-452 is available from the American Type Culture Collection (ATCC) under ATCC Number HB-11485. A copy of an information sheet for the antibody is attached herewith. This document was obtained from www.atcc.org. As indicated on the document, the hybridoma may be purchased from ATCC. The purified antibody can also be purchased from Pharmingen (as noted on page 38, line 14-15 of the specification). A copy of the technical data sheet for the antibody which was obstained from the BD Biosciences website is attached herewith. Thus, the antibody is publicly available and this rejection is moot.

Rejections under 35 U.S.C. §102

Sackstein et al.

Claims 1-7 were rejected under 35 U.S.C. §102(b) as anticipated by Sackstein et al. (*Blood*, 89:2773-2781, 1997; "Sackstein") as further evidenced by Dimitroff et al. (*J. Biol. Chem.*, 276:47623-47631, 2001; "Dimitroff"). The Examiner stated

> Sackstein et a. teach a hematopoietic cell L-selectin ligand which exhibits sulfate-independent binding activity that appears to be the same KG1a CD44 glycosylated polypeptide of the claimed invention...In further evidence, Dimitroff et al. discloses that the L-selectin ligand disclosed in Sackstein et al. reads on the instant hematopoietic cell E- and L-selectin ligand (see reference 18 cited in the Introduction, particularly page 47623, column 2, paragraph 1)...The claimed functional limitations would be inherent properties of the referenced KG1a L-selectin ligand.

This rejection is respectfully traversed. Claims 1-7 are directed to substantially purified preparations of glycosylated polypeptides comprising an amino acid sequence at least 95% identical to SEQ ID NO: 1 or to a sequence of a CD44 isoform arising from alternative splicing, which bind to an antibody having the binding specificity of monoclonal antibody HECA-452 and which comprise less than 30% of a polypeptide other than the glycosylated CD44 polypeptide. Sackstein does not teach a substantially purified polypeptide related to CD44. Sackstein shows that the L-selectin binding activity of the human hematopoietic cell line KG1a is not due to sulfation-dependent interactions. The characterization of the molecule responsible for L-selectin binding activity is limited; the reference neither identifies a polypeptide responsible for the L-

selectin binding activity of KG1a cells, nor discloses an agent capable of detecting and/or purifying the polypeptide. In the last sentence of the reference, it states

> Present efforts are directed at isolating and characterizing the structure of [the L-selectin ligand on KG1a cells]. Although the precise structural features that direct binding activity for this and other naturally expressed membrane L-selectin ligands remain to be determined, the data presented here demonstrate that determinants conferring high-affinity recognition of L-selectin may vary among different cell types that express such ligands.

Thus, the reference does not disclose the ligand(s) responsible for L-selectin binding activity, nor had it shown that it could be substantially purified.

The Examiner cited Dimitroff as evidence "that the L-selectin ligand disclosed in Sackstein et al. reads on the instant hematopoietic cell E- and L-selectin ligand." To establish inherency, extrinsic evidence must make clear that the missing descriptive matter (i.e., in the primary reference) is necessarily present in the prior art, and would be so recognized by persons of ordinary skill. As discussed above, Sackstein did not disclose any specific, substantially purified ligand. Dimitroff cannot substitute for this deficiency. Dimitroff's citation of Sackstein on page 47623, column 2, paragraph 1, is not evidence that Sackstein disclosed Applicant's claimed compositions. It is merely noting that HCELL is expressed on KG1a cells. Therefore, Sackstein does not anticipate Applicant's claims.

Stamenkovic et al.

Claims 1-7 were rejected as anticipated by Stamenkovic et al. (EMBO J., 10:343-348, 1991; "Stamenkovic") as evidenced by Sackstein (U.S. Pat. Pub. No. 2003/0040607; "US 2003/0040607"). The Examiner stated

> Stamenkovic et al. teach hematopoietic and endothelial forms of CD44, including encoding nucleotide and amino acids of CD44, which appear to the [*sic*] same or nearly the same as the instant hematopoietic cell L-selectin/E-selectin ligand...as set forth in Sackstein (US 2003/0040607 A1)...Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced CD44 glycoproteins...if the prior art teaches the identical chemical structure, the properties applicant discloses and/or claims are necessarily present.

This rejection is respectfully traversed. Stamenkovic does not disclose compositions have "identical chemical structure" to Applicant's claimed compositions, e.g., compositions comprising a substantially purified glycosylated polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO: 1 or to a sequence of a CD44 isoform arising from alternative splicing, which binds to an antibody having the binding specificity of monoclonal antibody HECA-452, and which comprise less than 30% of a polypeptide other than the glycosylated CD44 polypeptide. Furthermore, US 2003/0040607 does not indicate that the polypeptides disclosed by Stamenkovic are "necessarily" identical to Applicant's polypeptides.

Stamenkovic isolated a cDNA encoding a form of CD44 naturally expressed in endothelial cells, and compared the properties of the polypeptide encoded by this cDNA to a form naturally expressed in hematopoietic cells. The authors observed that the hematopoietic form, when expressed by transfection of Burkitt lymphoma cells (a B cell line) mediated binding to high endothelial cells (HEV), whereas the endothelial form (also expressed by transfection of Burkitt lymphoma cells) did not. Stamenkovic did not examine the glycosylation of the forms of CD44 expressed in his experiments, nor did he show that these the forms of CD44 bind an antibody having the binding specificity of HECA-452. US 2003/0040607 does not show that "the claimed functional limitations would be inherent properties of the referenced CD44 glycoproteins." If anything, US 2003/0040607 indicates that the molecules expressed in a B cell line by Stamenkovic are distinct from the claimed compositions. When a panel of cells including B cells were examined for HECA-452 reactivity of CD44 molecules, "only CD44 from CD34+ cells stained with HECA-452 and functioned as an E-selectin ligand." (paragraph 197 of US 2003/0040607). Thus, B cells were not observed to express HECA-452-reactive CD44 polypeptides in these experiments. There is no indication that the molecules examined by Stamenkovic are "necessarily" those of Applicant's compositions. Furthermore, Stamenkovic did not substantially purify CD44 polypeptides having the properties of the claimed polypeptides, such as E-selectin and/or L-selectin binding activity. Stamenkovic's experiments do not disclose or even suggest compositions having the features of Applicant's claims. Thus,

the reference fails to disclose a structure identical to a glycosylated polypeptide that binds to an antibody having the specificity of HECA-452.

U.S.S.N. 09/619,290

The Examiner provisionally rejected claims 1-7 under the doctrine of obviousness-type double patenting over copending application U.S.S.N. 09/619,290. Applicant will defer substantive response until the claims of one of the applications are allowed.

Enclosed is a $475 check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 23 Sept 2004

for Louis Myers No 55,658
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Cell Lines

ATCC Number:	**HB-11485** Order this item	**Price:**	**$290.00**
Designation:	HECA-452	**Depositors:**	Stanford University
Biosafety Level:	1 **Shipped:** frozen	**Isotype:**	IgM
Medium & Serum:	See Propagation	**Growth Properties:**	suspension
Organism:	*Rattus norvegicus* (B cell); *Mus musculus* (myeloma) (rat (B cell); mouse (myeloma))	**Morphology:**	lymphoblast
Tissue:	spleen; B lymphocyte; hybridoma		
Cellular Products:	immunoglobulin; monoclonal antibody; against human cutaneous lymphocyte antigen (CLA)		
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Related Cell Culture Products

Comments:	The antibody reacts with an antigen on skin homing memory T cells. It binds a T cell carbohydrate ligand for vascular E-selectin. The antibody also recognizes sialyl Lewis x and sialyl Lewis a antigens and other carbohydrate ligands for human E-selectin.
Strain:	Wistar
Propagation:	**ATCC complete growth medium:** RPMI 1640 medium with 0.05 mM 2-mercaptoethanol and non essential amino acids, 90%; fetal bovine serum, 10% **Temperature:** 37.0 C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 cells/ml and maintain between 1 X 10 exp5 and 1 X 10 exp6 cells/ml.
Freeze Medium:	
References:	22056: Butcher EC , Picker LJ . Neutrophil LECAM-1 as indicator of neutrophil activation. US Patent

5,316,913 dated May 31 1994
23183: Duijvestijn AM , et al. High endothelial differentiation in human lymphoid and inflammatory tissues defined by monoclonal antibody HECA-452. Am. J. Pathol. 130: 147-155, 1988. PubMed: 3276207
23441: Berg EL , et al. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. J. Exp. Med. 174: 1461-1466, 1991. PubMed: 1720810

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Technical Data Sheet

FITC Conjugated Rat Anti-human CLA

Product Information

Catalog Number:	**555947**
Size:	100 tests
Clone:	HECA-452
Isotype:	Rat IgM, κ
WS No.	V S075
Storage Buffer:	Aqueous buffered solution containing BSA* and 0.09% sodium azide. *Source of all serum is from the United States.

Description

Reacts with cutaneous lymphocyte associated antigen (CLA), a carbohydrate domain shared by sialyl Lewis^x (sLe^x) and sialyl Lewis^a (sLe^a) antigens. It serves as the ligand for selectins including CD62E (ELAM-1) and CD62L (LECAM-1). CLA is expressed on lymphocytes in the skin and on high endothelium. In peripheral blood, it is expressed on a small subset of lymphocytes and all monocytes and granulocytes. It has been suggested that CLA plays a role in supporting lymphocyte migration to extravascular tissues during inflammation.[1-5]

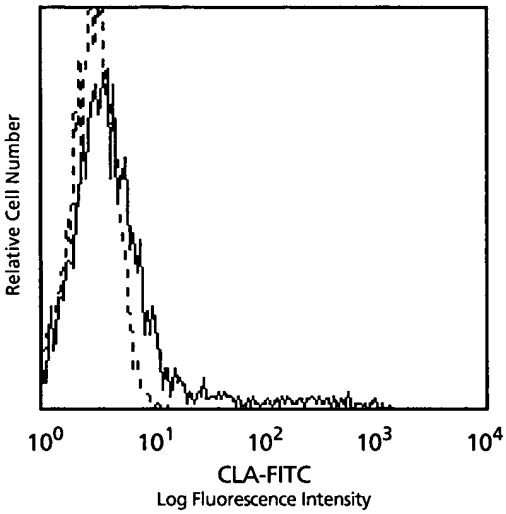

Figure 1. *Profile of peripheral blood lymphocytes analyzed by flow cytometry.*

Preparation and Storage

The antibody is purified from tissue culture supernatant by affinity chromatography and is conjugated with FITC under optimum conditions. The solution is free of unconjugated FITC.

Store conjugate at 4° C and avoid prolonged exposure to light. **Do not freeze.**

Usage

This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 20 µl/10^6 cells. Since applications vary, each investigator should titrate the reagent to obtain optimal results. **Note:** this product is routinely tested on peripheral blood lymphocytes. The histogram is an example of the expected reactivity.

Recommended Isotype Control: Rat IgM, κ, clone R4-22, Cat. No. 555951.

References

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5. Duijvestijn AM, Horst E, Pals ST, et al. High endothelial differentiation in human lymphoid and inflammatory tissues defined by monoclonal antibody HECA-452. *Amer. J. Pathol.* 1988;130:147.

Caution: *Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.*

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